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A Novel Cell-Surface Marker Found on Human Embryonic Hepatoblasts and a Subpopulation of Hepatic Biliary Epithelial Cells

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ABSTRACT

The nature of the cells that contribute to the repopulation of the liver after hepatic necrosis or cirrhosis remains uncertain, in part because we lack specific markers to facilitate identification and prospective isolation of progenitor cells. The monoclonal antibody GCTM-5 reacts with a minority subpopulation of cells in spontaneously differentiating cultures of pluripotent human embryonal carcinoma or embryonic stem cells. The epitope recognized by GCTM-5 is found on a 50-kDa protein present on the surface of these cells. In tissue sections of first-trimester human embryos, GCTM-5 specifically stained hepatoblasts and no other cell type examined. In normal pediatric or adult liver, GCTM-5 reacted with a minority population of luminal bile duct cells. In diseased livers, the numbers of GCTM-5–positive cells were increased compared with normal liver; antibody staining was restricted to a subpopulation of ductular reactive cells, and among this subpopulation we observed GCTM-5–positive cells that did not express cytokeratin 19 or N-CAM, classical makers of ductular reactive cells. Live GCTM-5–positive cells could be isolated from diseased livers by immunomagnetic sorting. These results suggest that GCTM-5 will be a useful reagent for defining cell lineage relationships between putative progenitor populations in embryonic liver and in the biliary epithelium during tissue repair. STEM CELLS *2005;23:103–112*

INTRODUCTION

Despite many years of study, there is still controversy over the nature of the stem cell or stem cells that contribute to the repopulation of the adult liver after certain forms of injury. Although it is clear that hepatocytes themselves account for much of the remarkable repopulation that follows partial hepatectomy [1], in other forms of injury, such as chemically induced damage, various authors have argued that oval cells, bile duct cells, or bone marrow–derived cells might represent facultative stem cell populations involved in repair and regeneration of the tissue [2]. Much evidence indicates that bone marrow–derived cells can participate in the repair of damaged liver tissue [3–5], and there is controversy over the extent to which cell fusion accounts for this apparent transdifferentiation [6–8]. However, a recent study of a particular chemically induced liver damage mouse model revealed that hepatic oval cells of the mouse do not originate from the bone marrow but from the liver itself [9]. The same study showed that oval cells did not originate from the dedifferentiation of mature hepatocytes and that these

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oval cells were at least as efficient as mature hepatocytes in repopulating the damaged liver.

Adult liver stem cells are also thought to reside in the terminal bile ductules. Oval cells are considered by some workers to be the daughter cells of these true stem cells, and these cells can also colonize the terminal bile ductules (known as the canals of Hering [10]) and the intrahepatic biliary system. In vitro and in vivo animal studies have suggested that these oval cells are bipotent and can differentiate to hepatocytes and bile ductular cells to repopulate injured liver [11]. Studies of diseased liver tissue have provided evidence for the presence of oval cells in humans. Morphological and immunohistochemical analysis of liver from patients with sub massive hepatic necrosis and cirrhosis attributable to hepatitis, alcohol injury, or primary biliary cirrhosis (PBC) revealed evidence of oval cell proliferation [12–14]. These oval cells were shown to express markers for biliary (cytokeratins [CKs] 7 and 19) and hepatocytic (alpha-fetoprotein and HepPar1) differentiation, suggesting the bipotential status of these cells. Ductular reactive cells also appear at the periportal margins during the disease process and express biliary markers and neuroendocrine features such as N-CAM and chromogranin A [15, 16]. The developmental relationship of oval cells to ductular reactive cells has not been fully evaluated.

There is at present a paucity of cell-surface markers to distinguish putative epithelial stem cell populations in the liver bile duct [17]. It is possible that certain aspects of liver cell regeneration might recapitulate embryonic development of the organ. In human embryogenesis, the first morphological sign of liver development is when the floor of the ventral foregut endoderm thickens to form the liver diverticulum at 3–4 weeks of gestation [18]. Hepatoblasts from the liver bud migrate in a cord-like fashion from the diverticulum into the surrounding loose mesenchym of the septum transversum and subsequently form the definitive liver [19]. It is then that the liver begins its early function as a site of hematopoiesis, and concurrently the bipotential hepatoblasts differentiate to mature hepatocytes and biliary cells. It is often suggested that signaling from the mesoderm is involved in the induction of early liver development. Recently it was demonstrated that initiation of mammalian liver development from endoderm is influenced by fibroblast growth factors, expressed by the adjacent cardiac mesoderm [20]. Furthermore, it was shown that different fibroblast growth factors (FGFs), FGF-1, -2, and -8, are involved in initiating distinct phases of mammalian hepatogenesis, a process of hepatocyte and duct maturation. The intrahepatic bile duct system begins to form around the eighth week of gestation, when hepatoblasts are induced to form a sleeve of cells (the ductal plate) around the portal vein. Bile ducts are formed from this ductal plate [21].

Germ cell tumors of the testis are developmental neoplasms that arise during embryogenesis from the precursors of sperm and egg known as primordial germ cells. The stem cells of these tumors are pluripotent [22]. Cultured cell lines have been derived from testicular germ cell tumors in humans, and they are capable of differentiation into a wide variety of human adult tissue in vitro and in vivo when xenografted into immunosuppressed mice. Thus, cell cultures derived from embryonal tumors, when undergoing differentiation, will contain a range of cell types characteristic of different stages of development of many tissues of the human embryo, fetus, and adult organs, and these differentiating cultures may be used to discover cell lineage and developmental stage–specific markers.

GCTM-5 is a monoclonal antibody originally derived after immunization of mice with a membrane preparation from a testicular seminoma. Screening of this reagent against a human embryonal carcinoma cell line revealed that it bound to a minority cell population in the culture that was unreactive with markers of pluripotent stem cells. In this study, we show that GCTM-5 is expressed exclusively in the fetal liver of 7-week human embryos. Concurrent experimentation on normal and diseased pediatric and adult human liver tissue reveals an expression localized to a subpopulation of cells within the biliary epithelium.

Materials and Methods

Fetal, Pediatric, and Adult Tissue

Histological sections of first-trimester human embryos were cut from archival material obtained with informed consent from patients undergoing termination of pregnancy at the John Radcliffe Hospital, Oxford, U.K. The tissue was fixed in absolute alcohol, embedded in paraffin, and sectioned at 5-μM thickness. Liver tissue was obtained from the adult and pediatric liver transplant programs at the University & Birmingham Children's Hospitals, NHS Trust Birmingham, U.K. Hepatectomy specimens were obtained from patients with PBC ($n = 6$), alcoholic liver cirrhosis (ALD) ($n = 6$), and extrahepatic biliary atresia (EHBA) ($n = 3$). Donor tissue was obtained from the pediatric transplant program when in excess to surgical requirements and served as normal control ($n = 5$). For immunohistochemistry, tissue was snap frozen and stored at –70°C. For cell isolation, tissue was stored in Dulbecco's modified Eagle's medium at 4°C and used within 48 hours after hepatectomy. All tissue was obtained following local ethical committee approval and with informed patient written consent.

Cultured Cell Lines

Cell lines GCT27X-1 (human embryonal carcinoma), GCT 44 (primitive endoderm yolk sac carcinoma), GCT 72, and GCT119 (visceral yolk sac carcinoma) were cultured as described elsewhere [23, 24]. Human embryonic stem (ES) cell lines HES-2 and HES-3 were propagated as described by Reubinoff et al. [25], and differentiated cells were obtained by cultivation of colonies to high density in situ [25] or by modification of the culture medium (Hawes, Gion, and Pera, unpublished data). Cell lines HEK293 and HepG2 were propagated as monolayer cultures in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics.

Derivation and Characterization of GCTM-5 Antibody

Female Balb/C mice 4 – 6 weeks of age were immunized with a crude membrane preparation from a testicular seminoma. Fresh tumor tissue was disrupted by Dounce homogenization, and the homogenate was subjected to low-speed centrifugation at 3,000*g* to remove nuclei, mitochondria, cytoskeletal elements, and debris and then subjected to high-speed centrifugation at 100,000*g* for 1 hour. The resulting pellet was used for immunization. Fusion with myeloma cell line NS-1 and subsequent selection of hybridomas was carried out using standard techniques. Hybridoma supernatants were first screened by antibody capture enzyme-linked immunosorbent assay using the immunizing membrane preparation. Secondary screening was performed using either immunocytochemistry on fixed or frozen sections of seminomas or by indirect immunofluorescence on fixed slides of cultured embryonal carcinoma cell lines, including GCT27X-1.

Indirect Immunofluorescence

GCT27X-1 embryonal carcinoma cells or human ES cells grown on 12-well slides were fixed with 100% ethanol. For indirect immunofluorescent analysis of differentiating human embryonal carcinoma or ES cell cultures, a combination of primary antibody GCTM-5 (immunoglobulin H1 [IgG1], neat supernatant) and fluorescein-conjugated secondary antibody, anti-mouse Ig–fluorescein isothiocyanate (FITC) (1:40, Dako, Sydney, New South Wales, Australia) was used. Cell nuclei were then stained with 1 μg/ml 4',6-diamidino-2-phenylindole dihydrochloride (Sigma, Sydney, New South Wales, Australia).

Double immunostaining studies of antigen colocalization in normal and diseased adult liver tissue was performed using the fluorescent conjugate Texas Red (IgG1) or FITC (IgG2a) (Cambridge BioScience, Cambridge, U.K.) on 5-μm cryostat sections fixed in acetone as described previously [16]. The primary antibody combination consisted of anti-CK-19 (IgG2a, 1:10, Progen, Heidelberg, Germany) or anti-N-CAM (IgG2a, Dako) with GCTM-5 (IgG1, neat supernatant).

Cell-Surface Localization of GCTM-5 Antigen

Immunostaining of live differentiating ES cell cultures 5 weeks after subculture was achieved by addition of the primary antibody to the cells before fixation. The cells were incubated with GCTM-5 antibody for 10 minutes at 4°C. Then cells were gently washed with phosphate-buffered saline three times and fixed in cold 100% ethanol for 5 minutes. A 1:50 dilution of anti-mouse Ig conjugated to alkaline phosphatase (Dako) was then added, the sections were washed after 30 minutes in Tris-buffered saline (25 mM Tris, 150 mM NaCl, pH 8), and visualization of the secondary antibody was achieved using the SIGMA *FAST*: Fast Red TR/Naphthol AS-MX (Sigma). The sections were counterstained with Mayers' hemotoxylin.

Staining of Human Fetal Sections

Paraffin-embedded human fetal sections from three separate specimens were dewaxed by two 5-minute incubations in the citrus-based clearing agent Histolene (Merck, Melbourne, Victoria, Australia). The tissue was cleared and rehydrated with two 5-minute incubations in 100% ethanol, followed by 5-minute incubation in 70% ethanol and 5 minutes in running water. The sections were incubated with neat GCTM-5 supernatant before addition of an anti-mouse Ig conjugated to alkaline phosphatase, which was detected as described above.

Immunoblot Analysis of GCTM-5 Antigen

Differentiating cultures of human embryonal carcinoma or ES cell lines were grown in 12-well tissue culture plates. Cells in four wells of a plate were lysed with Laemmli sample buffer containing 0.2 M dithiothreitol. A sample of cultured biliary epithelial cells, isolated from a patient with primary biliary cirrhosis using antibody HEA-125 and maintained in primary culture for 10 days (below), was also lysed into Laemmli sample buffer. The samples were run on a 10% sodium dodecyl sulphate polyacrylamide gel with a BENCHMARK Prestained Protein Ladder (Invitrogen, Melbourne, Victoria, Australia). The protein was then transferred to Hybond-P membrane (Amersham Biosciences, Melbourne, Victoria, Australia), and the membrane was blotted with GCTM-5 (neat) or CAM5.2 against CK8 and CK18 (BD Biosciences, Melbourne, Victoria, Australia) as a positive control. An anti-mouse Ig conjugated to horseradish peroxidase secondary antibody (Dako) was added at a dilution of 1:10,000 in Tris-buffered saline-Tween 20. Chemiluminescent ECL Reagent

(Amersham Biosciences) was added to the membrane for 5 minutes before exposure of the membrane to Hyperfilm (Amersham Biosciences).

Diseased Liver Cell Isolation and Culture

Isolation of HEA-125–positive and GCTM-5–positive cells was based on methodology previously described [26, 27]. Briefly, after Percoll density gradient centrifugation at 800*g* for 30 minutes, the nonparenchymal fraction at the Percoll band interface and the 1.04 g ml^{-1} layer were removed. The suspension was divided into two equal fractions, and cells were additionally purified using immunomagnetic separation with either the biliary cell marker human epithelial antigen-125 (HEA-125) (1:10, IgG1, Progen) or GCTM-5 antibody. The antibody-coated cells were then selected using magnetic Dynabeads (subclass IgG, Dynal, Wirral, U.K.).

Isolated cells from each of the fractions were resuspended in biliary plating media [28] and plated in $12 \times 2\ cm^3$ wells (24-well plates) and incubated at 37°C with 5% CO_2. After 24 – 72 hours, media were removed and replaced with biliary cell growth media [28] and re-fed on alternate days.

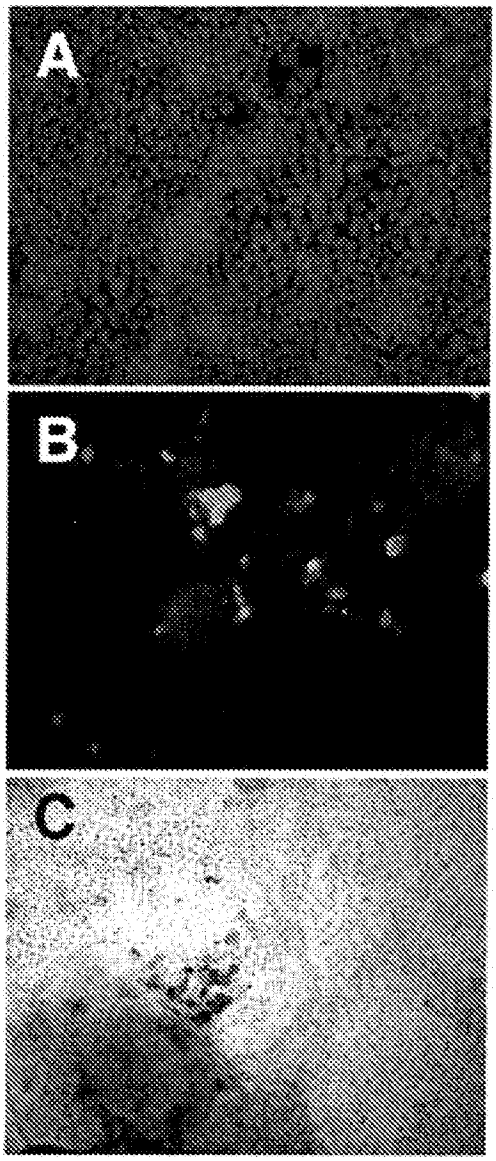

Figure 1. Expression of GCTM-5 in differentiating cultures of human pluripotent stem cells. **(A, B)**: Embryonal carcinoma cell line GCT 27-X1. **(A)**: DNA staining. **(B)**: GCTM-5 immunostaining. **(C)**: Staining of live cultures of differentiating human embryonic stem cells (HES-3) with GCTM-5; cluster of positive cells in red, hematoxylin counterstain in blue. Magnification × 100 on all panels.

Phenotypic Characterization of Cultured Cells

After 6 days, cultured cells were stained for expression of specific proteins. The cells were fixed with 70% vol/vol ethanol and washed twice with phosphate-buffered saline (pH 7.4). The following primary antibodies were incubated for 1 hour at 25°C: CK-19 (IgG1, 1:100, DAKO, High Wycombe, U.K.) and HEA-125 (IgG1, 1:100), both specific for biliary epithelial cells in liver; CK-18 (IgG1, 1:10, DAKO), recognizing both hepatocytes and biliary epithelial cells; CD31 (1:100, Dako), a marker for endothelial cells; and GCTM-5 (IgG1, neat). Staining was visualized using the immunoperoxidase Vector Stain ABC Elite kit (Vector Labs, Peterborough, U.K.).

Results

Summary of GCTM-5 Expression in Cultured Cell Lines and Isolated Cells

A panel of cell lines was examined for expression of GCTM-5 antigen using indirect immunofluorescence. Reactivity of the antibody was first noted with a small subpopulation of differentiated cells in cultures of human embryonal carcinoma cell line

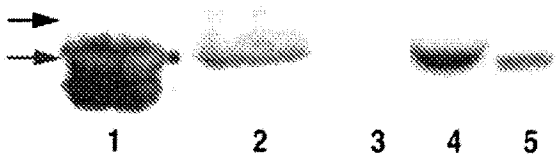

Figure 2. Immunoblot of a whole-cell lysate of differentiating culture of human embryonal carcinoma cell line GCT 27X-1 probed with GCTM-5, CAM 5.2 against CK-8 and -18, or secondary antibody only or lysates of biliary epithelial cells and differentiating ES cells probed with GCTM-5 . Lane 1, CAM 5.2; lane 2, GCTM-5; lane 3, secondary antibody only; lane 4, biliary epithelial cell lysate probed with GCTM-5; lane 5, differentiating human ES cell lysate probed with GCTM-5. Arrows indicate position of 64- and 49-kDa molecular weight markers. Abbreviations: CK, cytokeratin; ES, embryonic stem.

GCT 27X-1. Table 1 shows a summary of the reactivity of the antibody with cultured cell lines, representing an adult hepatocarcinoma cell line, immortalized human embryonal kidney cells, embryonal carcinoma, yolk sac carcinoma, human ES cells, and primary biliary epithelial cells. Positively stained cells showing a characteristic polygonal morphology were observed consistently in differentiating cultures of human embryonal carcinoma or ES cells; other permanent cell lines were negative or showed only sporadic reactivity. Figures 1A and 1B show DNA staining and immunostaining, respectively, of a culture of GCT27X-1 cells with GCTM-5. Fixed preparations of differentiating cultures of ES cells showed a similar staining pattern in a small minority (<1%) of cells (not shown). Incubation of live cultures of differentiating ES cells at 4°C with the antibody before fixation revealed that the epitope reactive with the reagent was indeed accessible outside of the cell (Fig. 1C). The nature of the GCTM-5–positive cells within the human ES cell cultures will be the subject of a separate communication.

Immunoblotting of GCTM-5 Antigen

Immunoblot analysis of a whole-cell lysate of differentiated human embryonal carcinoma stem cells revealed a GCTM-5 protein band of approximately 50 kDa (Fig. 2, lane 2). Lanes probed with antibody CAM 5.2 showed bands of the expected sizes of 50 and 43 kDa (Fig. 2, lane 1), whereas the lane probed with only the secondary antibody showed no staining (Fig. 2, lane 3). Lysates of biliary epithelial cells from a patient with primary biliary cirrhosis (Fig. 2, lane 4) or differentiating human ES cells (Fig. 2, lane 5) also contained bands of 50-kDa reactive with antibody GCTM-5.

GCTM-5 Expression Analysis in the 7-Week Human Embryo

The presence of GCTM-5–positive cells in differentiating cultures of human ES cells suggested that such cells might be found in the developing human embryo. To screen for expression of the marker during human development, we examined sections of first-trimester embryos. GCTM-5 was expressed exclusively in the liver of the 7-week human embryo in hepatoblasts but not hematopoietic cells, as shown in Figure 3A. GCTM-5 staining was not detected in fetal heart, lung, kidney, central nervous system, and definitive gut (gut and neural tissue; Figs. 3C, 3D). Significantly, sections of human yolk sac failed to show reactivity with GCTM-5 (data not shown).

GCTM-5 Expression in Normal and Diseased Liver

In normal liver (age 5 – 34 years), GCTM-5 was present on the luminal surface of bile ducts and colocalized with the biliary cell marker CK-19 (Fig. 4A). Many CK-19–positive cells were negative for GCTM-5. In diseased liver, including PBC and ALD, where some intact bile ducts remained, colocalization with CK-19 and GCTM-5 was again apparent on the luminal aspect of some of the bile ducts (Fig. 4B, PBC). The pattern of GCTM-5 staining on ductular reactive cells in diseased tissue was particularly noteworthy. GCTM-5 seemed to label only a subpopulation of CK19-positive cells (Figs. 4C, 4D). Moreover, a small but discrete number of cells were positive for GCTM-5 only (Figs. 4B, 4C). Further immunostaining for N-CAM, another marker reported to label ductular-reactive cells specifically (and not cells in intact

Table 1. GCTM-5 expression analysis in a variety of cultured cell lines and isolated biliary cells

Cell line	Cell line description	GCTM-5 expression
HepG2	Hepatocellular carcinoma	–
HEK293-T	Human embryonal kidney	–
GCT27X-1	Human embryonal carcinoma	+
GCT44	Primitive endoderm-like yolk sac carcinoma	–
GCT72	Visceral endoderm-like yolk sac carcinoma	+/– (occasional positive cells in some cultures)
GCT119	Visceral endoderm-like yolk sac carcinoma	–
HES-2 HES-3	Differentiating cultures of human embryonic stem cells	+
Bilary epithelial cells	–	+++

GCTM-5 expression: –, no cells stained; +, clear positive staining in <1% of cells; ++, strong expression in majority of cells.

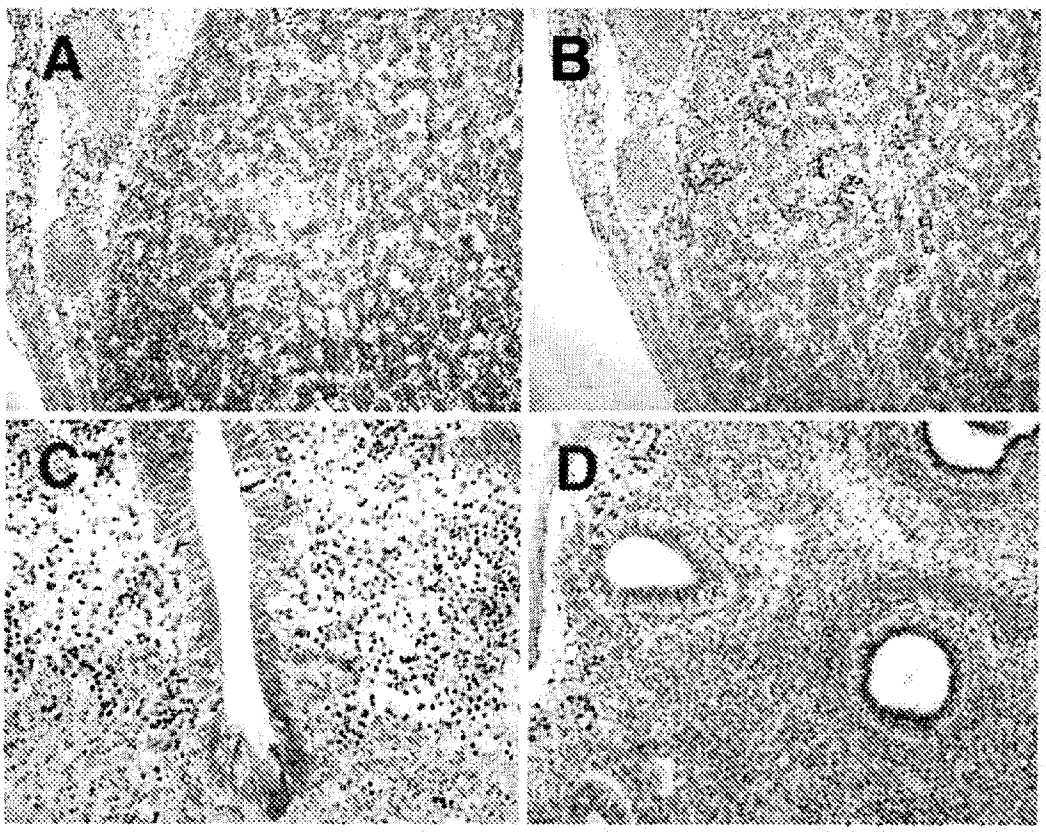

Figure 3. Immunohistochemical staining of human embryonic tissue (7 weeks of gestation) with GCTM-5. (**A**): Liver stained with GCTM-5. (**B**): Control for (**A**) (no primary antibody). (**C**): Neural tube stained with GCTM-5. (**D**): Gut loops stained with GCTM-5. Magnification ×100 (**A, B**) and ×200 (**C, D**).

ducts), revealed a similar pattern of expression. N-CAM displayed some colocalization with GCTM-5, but not all cells were colabeled, and there were individual cells positive for GCTM-5 only (Fig. 4E).

Phenotype of GCTM-5–Cultured Cells from Cirrhotic Liver

Because GCTM-5 seemed to recognize a cell-surface epitope, liver cells were isolated using immunomagnetic separation and grown in culture for up to 6 days. The phenotype of these cells was compared with biliary epithelial cells isolated using HEA-125 from four diseased livers (three PBC and one ALD). With time in culture in biliary growth medium, colonies expanded in both groups and gave rise to cultures with similar properties. When immunostained using the biliary cell markers CK-19 or HEA-125, colonies derived from the isolated GCTM-5 and HEA-125 cells were positive (Figs. 5A–5D). All cultured cells stained with CK-18 (data not shown). Both groups of cultured cells stained positive for the GCTM-5 antibody (Figs. 5E, 5F) with some variation in the level of reactivity, but both were negative for the endothelial cell marker CD31 (Figs. 5G, 5H) and no primary control (data not shown).

Discussion

The finding that the GCTM-5 antigen is found on a subpopulation of cells present in differentiating cultures of human embryonal carcinoma and ES cells suggested that the surface marker might be restricted to specific early cell lineages in the mammalian embryo. Indeed, immunocytochemistry on the 7-week human embryo revealed that GCTM-5 is expressed exclusively in the liver. GCTM-5 uniformly stained hepatoblasts, the bipotential cells of the fetal liver that differentiate to give rise to both hepatocytes and biliary cells. This suggests that GCTM-5 recognizes an early liver progenitor population and indicates GCTM-5 could be a useful tool for the further study of early human embryogenesis, in particular liver formation. There is much overlap in gene expression between the yolk sac and the liver. Therefore, the absence of significant reactivity of GCTM-5 with yolk sac carcinoma cell lines or with the normal human yolk sac indicates that the antibody will be a useful marker for distinguishing between extraembryonic cells and embryonic endodermal cells in differentiating cultures of human ES cells.

The liver possesses a stem cell population that is at least bipotent and is activated by massive liver necrosis

or cirrhosis, conditions in which regeneration occurs but hepatocyte proliferation may be blocked. These liver stem cells are considered by some to reside in the terminal bile ductules [10, 29].

GCTM-5 staining of diseased liver of patients with PBC, EHBA, or ALD revealed localization to the ductular reactive cells of these tissues. Staining in normal pediatric and adult liver showed a much reduced expression of GCTM-5 compared with diseased tissues, which was restricted to the luminal surface of some mature bile ducts. It is also interesting to note that in almost all cases of diseased tissue examined, discrete GCTM-5–positive cells that did not express the biliary/ductal reactive cell markers CK19 and N-CAM were found adjacent to GCTM-5–positive cells that did express these markers. N-CAM has previously been reported to recognize ductular-reactive

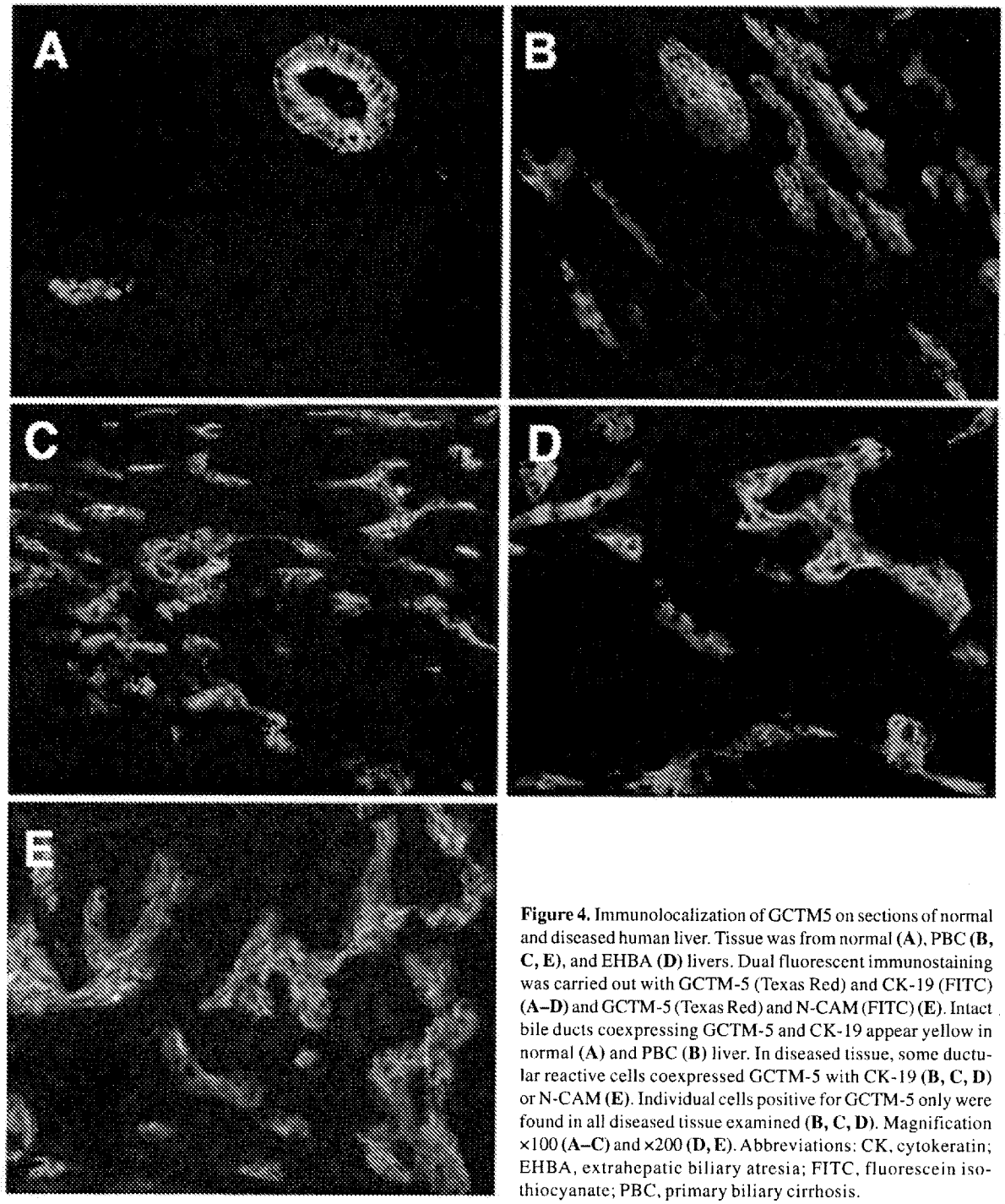

Figure 4. Immunolocalization of GCTM5 on sections of normal and diseased human liver. Tissue was from normal **(A)**, PBC **(B, C, E)**, and EHBA **(D)** livers. Dual fluorescent immunostaining was carried out with GCTM-5 (Texas Red) and CK-19 (FITC) **(A–D)** and GCTM-5 (Texas Red) and N-CAM (FITC) **(E)**. Intact bile ducts coexpressing GCTM-5 and CK-19 appear yellow in normal **(A)** and PBC **(B)** liver. In diseased tissue, some ductular reactive cells coexpressed GCTM-5 with CK-19 **(B, C, D)** or N-CAM **(E)**. Individual cells positive for GCTM-5 only were found in all diseased tissue examined **(B, C, D)**. Magnification ×100 **(A–C)** and ×200 **(D, E)**. Abbreviations: CK, cytokeratin; EHBA, extrahepatic biliary atresia; FITC, fluorescein isothiocyanate; PBC, primary biliary cirrhosis.

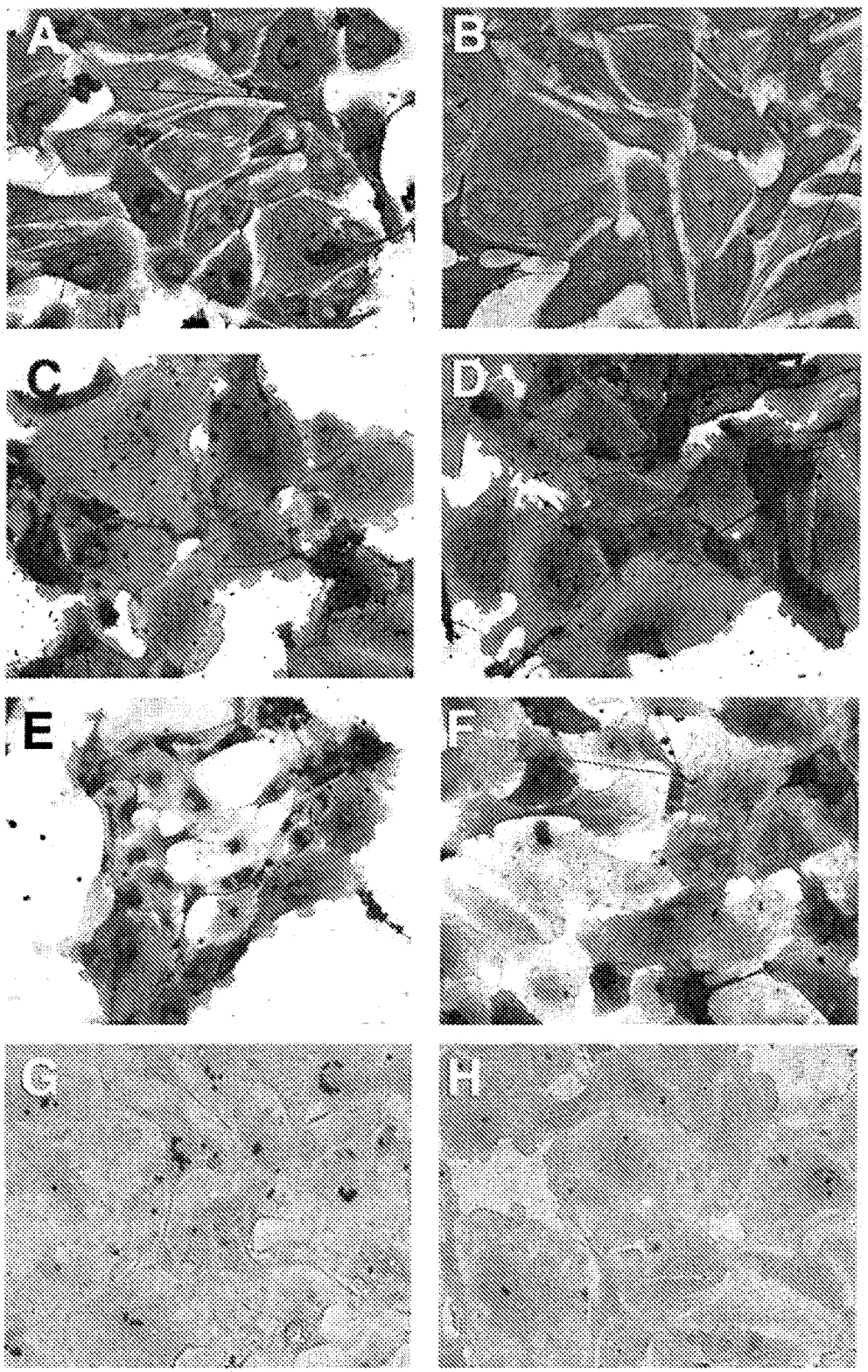

Figure 5. Immunocharacterization of HEA-125 (**A, C, E, G**) or GCTM5 (**B, D, F, H**) immunoisolated cells from PBC or ALD liver after 6 days in culture. The GCTM-5 and HEA-isolated cells both expressed a biliary epithelial cell phenotype (CK-19 and HEA positive) (**A–D**) after short-term culture. Both sets of cells were positive for GCTM-5 (**E, F**) and negative for the endothelial cell marker CD31 (**G, H**). Cells were isolated from PBC (**A, B, E–H**) and ALD (**C, D**). Magnification ×200. Abbreviations: ALD, alcoholic liver cirrhosis; PBC, primary biliary cirrhosis.

cells specifically and not biliary cells that are integrated into ducts [15, 16]. In the normal livers examined, there was no evidence of cells that were GCTM-5–positive and CK19-negative. Thus, GCTM-5 may identify a lineage progression of stem cells into ductular-reactive cells and biliary phenotype after recruitment in disease. However, our data do not rule out the interesting possibility that the GCTM-5–positive cells might in fact originate from the bone marrow.

The findings presented here strongly imply that GCTM-5 recognizes an epitope expressed on proliferating liver progenitor cells found predominantly in diseased, regenerating tissue. It is possible that these progenitors are a bipotent population with the ability to differentiate to mature biliary cells and hepatocytes, although evidence of expression of GCTM-5 on hepatocytes or hepatocyte-like cells has not yet been found. One of the factors that has limited progress on the question of the identity, localization, and potential uses of human liver-derived stem cells and cells of the canals of Hering has been the absolute lack of specific surface (or internal) markers. Although investigators often use antibodies against CK (notably CK-7/-19) as markers of oval cells or ductular reactive cells, their expression in mature biliary epithelium inevitably leads to lack of clarity. The marker currently regarded as the best, OV6 [13, 21], seems to recognize epitopes on CK [30] and cannot be used to immunoisolate live cells.

The potential for GCTM-5 to fill this gap is promising. GCTM-5 staining of live unpermeabilized cells demonstrated that GCTM-5 is a cell-surface marker, binding to pericellular epitopes. This important property enables immunoisolation of live cells for molecular, biochemical, and cell biological analyses. Immunocytochemical analysis of GCTM-5–positive isolated cells from PBC diseased liver revealed a similar phenotype to HEA-125 isolated cells [27]. GCTM-5–positive cells expressed mature biliary markers, CK19, epithelial membrane antigen, and HEA-125 but did not express CD31, an endothelial marker. However, it is important to note that these isolated liver cells were examined for marker expression after 6 days of culture in a system designed specifically to support maintenance of biliary epithelial cells. Thus, this experiment provides only limited information on the differentiation status and potential of GCTM-5–positive cells. The molecular weight of the GCTM-5 antigen shows that it is distinct from HEA-125, which reacts with the 34-kDa Ep-CAM [31]. Expression of GCTM-5 did not fully overlap with HEA-125, CK-19, or N-CAM in many biliary epithelial cells in normal or diseased livers, indicating that GCTM-5 recognizes distinct cell populations to these other biliary cell markers. Future studies will aim to isolate GCTM-5–positive cells from human ES cell cultures, from the developing liver, and from normal and diseased adult liver (including those cells that express other markers of ductular reactive cells and those that do not). This will enable additional investigation of the differentiation potential of the GCTM-5–positive cells from fetal or adult liver using transplantation assays and culture systems that support longer-term growth of multipotent hepatic progenitor cells to help elucidate the role of the GCTM-5–positive cell populations in liver development and disease.

Conclusions

Monoclonal antibody GCTM-5 reacts with a 50-kDa cell-surface protein expressed by differentiated cells in human ES cell cultures, embryonic hepatoblasts, and a minority population of biliary epithelial cells in the adult liver. In liver disease, the proportion of GCTM-5–positive cells is increased, but some GCTM-5–positive cells may be distinguished from biliary reactive cells. GCTM-5 will be a useful reagent for defining cell lineage relationships between putative progenitor populations in embryonic liver and in the biliary epithelium during tissue repair.

Acknowledgments

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Lincon Stamp, Heather Crosby, and Susan Hawes all contributed equally to this work.

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